Amendments to the Drawings

Figure 1

A spelling error was discovered in Figure 1 of the instant application. "Glucosamine" was spelled "glucoseamine." A replacement sheet with the correct spelling and a marked-up version of Figure 1 to show the changes made are in the attached sheets.

Attachment: Replacement Sheet
Annotated Sheet Showing Changes

REMARKS

Applicant's attorney thanks the Examiner for the teleconference of October 5, 2005. In this interview, patentability of a proposed amended claim set was discussed, vis a vis references cited by the Examiner and other references in the art.

Claims 1-41 are pending in the application. All claims have been rejected. An amended version of the claims is attached. Support for new claims 42 and 43 can be found on pages 22-23 and in Figure 3. Because Applicant is submitting herewith a petition for a one month extension under 37 CFR § 1.136, accompanied by an authorization to charge the appropriate fee to Deposit Account No. 09-0089, this response is timely filed. An information disclosure statement is also filed herewith.

Rejections under 35 USC § 102

Claims 1, 2, 7, 11, and 16 stand rejected under 35 USC § 102 as being anticipated by Goodwin et al., US Patent No. 5,858,783. Goodwin et al. discloses a method for culturing cells so as to form three-dimensional, tissue-like cell aggregates that employs a "culture matrix," which is preferably "5 mg/ml Cyodex-3 generally spherical microcarriers." US Patent No. 5,858,783, col. 5, lines 32-34, col. 9, lines16-21. Microcarriers are particularly apparent in Figures 2, 6, 8, and 10 of US Patent No. 5,858,783. Thus, the method of Goodwin et al. is a method for culturing of attached cells, not a method for culturing cells in suspension. The preferred culture medium of Goodwin et al. comprises glucose, galactose, and fructose.

Claim 1 in the instant application recites a method for controlling sialic acid content of a protein by culturing mammalian cells in a medium comprising galactose and fructose and has been amended to include the limitation of culturing the cells "in suspension," a limitation not described in Goodwin et al. Support for this amendment can be found on page 19, lines 21-22 of the specification. Hence claim 1, and dependent claims 2 and 7, are not anticipated by Goodwin et al.

Claim 11 recites a medium for culturing mammalian cells and has been amended such that the medium contains mannose and N-acetylmannosamine (ManNAc) in addition to galactose and fructose. Thus, claim 11 and dependent claim 16 are not anticipated by Goodwin et al.

Rejections under 35 USC § 103(a)

All claims have been rejected under 35 USC § 103(a) as being obvious over Franze et al. (US Patent No. 6,673,575) in view of Schnaar et al. (US Patent No. 6,274,568), Wood (US Patent No. 6,472,175), Gu et al. (1997), or Gu et al. (1997). Franze et al. teach culturing cells

in medium for the production of sialylated proteins and suggest the use of media that contain at least two or three different carbohydrates selected from a list of eleven sugars, which includes mannose, fructose, and galactose and does not include ManNAc. Hence, Franze et al. disclose a large genus of combinations of sugars, and species claims appropriately supported by data should be patentable over this disclosure. Only one combination of sugars is specifically called out in Franze et al., that is, glucose, galactose and mannose. This combination is used in Examples 5 (fermentation B), 6 (fermentations B and C), and 7 (fermentation F). However, none of these examples compare cultures that are identical except for the addition of mannose and galactose to one and not the other. Example 7 comes closest to this comparison. In this example, a culture in which glucose concentration was maintained at 3 ± 0.5 g/L by feeding with glucose is compared to a culture in which the concentration of all sugars was maintained in the range between 0.25 g/L and 3.5 g/L by feeding with glucose, galactose, and mannose in a mass ratio of 1:2:3. Example 5 compares a glucose-fed culture with a glucose-, mannose-, and galactose-fed culture. However, the feeding regimes are different. Thus, Franze et al. fail to demonstrate that the addition of galactose and mannose to a culture increases protein sialylation. Gu et al. (Biotechnol. Bioeng. 58: 642-48 (1998)) demonstrate that N-acetylmannosamine in culture medium can increase the sialylation of a protein produced by cultured cells. *See e.g.* Table II, page 645. Schnaar uses various carbohydrates, including N-acetylmannosamine, to control, not necessarily to increase, sialylation. Wood et al. describes using N-acetylmannosamine in an insect cell culture. Applicants assert that Wood et al. is not relevant to the instant invention because the instant invention is concerned with mammalian cells, not insect cells.

The reference Keppler et al. (submitted in a supplementary Information Disclosure Statement herewith) teaches away from the addition of sialic acid precursors upstream of UDP-GlcNAc 2-epimerase (which catalyzes the conversion of UDP-N-acetylglucose to N-acetylmannosamine; Fig. 1A in Keppler et al.) to increase sialylation. They demonstrate that supplementation of mammalian cell culture medium with N-acetylglucosamine, glucosamine, mannose, or glucose, all of which enter the sialic acid biosynthetic pathway upstream of UDP-GlcNAc 2-epimerase, have no effect no sialoglycan expression, while supplementation of medium with N-acetylmannosamine or mannosamine did increase sialoglycan expression. Galactose and fructose also enter the sialic acid biosynthetic pathway upstream of UDP-GlcNAc 2-epimerase. *See* Figure 1 of the instant application. Keppler et al. also observe a correlation between UDP-GlcNAc 2-epimerase activity and sialic acid content of cells and state that UDP-GlcNAc 2-epimerase is therefore "an important regulator of cell surface glycoconjugate sialylation in hematopoietic cell lines." Thus, Keppler et al. teaches away from the claimed methods and media.

Moreover, the findings of Keppler et al. are consistent with Applicant's finding that the addition of mannose to a mammalian cell culture (which includes glucose) does not enhance protein sialylation. Example 1, Figure 2. Further, Applicant's data show that the addition to a glucose-containing culture medium of some of the following sugars is also ineffective in increasing sialylation: (1) mannose; (2) mannose plus galactose; (3) fructose; (4) fructose plus mannose; (5) N-acetylmannosamine plus mannose; and (6) N-acetylmannosamine plus fructose.

A lack of a reasonable expectation of success in combining the cited references can be found in the complexity of the biochemical and physical processes involved in protein sialylation and other biochemical processes. Amounts of protein sialylation can vary among mammalian cells lines by at least four fold, indicating that significant variability exists among different cell lines with respect to protein sialylation. *See* Table 1 *in* Keppler et al. Stephanopoulous and Vallino (cited in accompanying Information Disclosure Statement) point out that researchers have focused on enzyme amplification or other modifications in the product pathway to enhance production of a specific metabolic product. They continue, "However, overproduction of many metabolites requires significant redirection of flux distribution in the primary metabolism, which may not readily occur following product deregulation because metabolic pathways have evolved to exhibit control architectures that resist flux alterations at branch points." Thus, knowledge of a sequence of steps in a biosynthetic pathway may not necessarily provide all information necessary to manipulate the pathway to achieve overproduction of a desired metabolite.

Uncertainty about whether a particular factor is limiting in the biosynthetic pathway that leads to sialylation is evinced in a 2005 publication of Wang et al. (submitted in an Information Disclosure Statement herewith). The authors investigate the effects of increasing levels of CMP-sialic acid transporter protein (which transports CMP-sialic acid from the cytoplasm into the Golgi, where protein sialylation takes place) and, in some cases, also feeding with ManNAc. They state, "This cell engineering approach to improve sialylation in CHO cells has not been known to be reported elsewhere. Depending on the CHO cell line and its recombinant protein product, this approach can be utilized singly or in combination with existing strategies, to overcome some of the present limitations that result in incomplete sialylation on recombinant proteins." Hence, according to these authors, previous work had not established whether increasing levels of CMP-sialic acid transporter protein would increase sialylation. In light of uncertainty in the art about whether overproduction of at least one enzyme needed for protein sialylation would affect sialylation, the effects of the addition of various sugars to a culture medium is difficult to predict.

A case for lack of obviousness of each of the amended independent claims is discussed below with reference to the arguments explained above. First, amended claim 1 is not obvious over the cited references because it recites a species with respect to Franze et al. and the result that galactose and fructose together enhance sialylation was unexpected in view of the complexity of the biosynthetic system and the teachings of Keppler et al., which teach away from the claimed invention. Further, the data in Figure 2 of the specification show that addition of galactose alone increased sialylation to some extent, and the addition of fructose increased sialylation further, even though the addition fructose alone had no effect on sialylation. Thus, the combination of galactose and fructose exhibited more than additive, i.e., synergistic, effects with respect to either alone.

Amended claims 11 and 33 are nonobvious with respect to the cited references because they recite a species with respect to Franze et al., and the results obtained with this combination are unexpected and synergistic. Although Franze et al. do recite media containing any of a large genus of combinations of sugars, including combinations comprising mannose, fructose, and galactose, it does not specifically call out the claimed combinations. In view of Keppler et al. and the complex nature of the process, the results obtained using a media comprising fructose, galactose, and mannose were unexpected and nonadditive (if the results of the addition of each sugar added singly are compared with the results of adding all three together). Figure 2.

Claims 19 and 27 are nonobvious with respect to the cited references because there was no expectation of success and the results were unexpected. In view of Keppler et al., the addition of galactose to medium would not be expected to increase sialylation. Results in the instant specification show that addition of galactose alone does increase sialylation but that addition of ManNAc plus galactose increases sialylation even more, even though the addition of ManNAc and either mannose or fructose actually decreased sialylation at the concentrations used. Figure 2. Thus, the success of this combination in increasing sialylation was unexpected.

Claim 41 is novel for all the reason stated above. In addition, claim 41 includes concentration limitations for fructose, galactose, and mannose. The data presented in Example 2 and Figure 3 show a concentration optimum for sialylation within the claimed range. Therefore, even if other arguments are not persuasive, the range limitations of claim 41 should make it patentably distinct, since the finding of a specific optimum was unexpected.

Conclusion

Applicant believes that all claims are in condition for allowance and respectfully requests notice to that effect. Should the Examiner believe that any issue can be most

expeditiously handled via teleconference, he is invited to telephone the undersigned at the direct dial number below.

Respectfully submitted,

Rosemary Sweeney

Rosemary Sweeney
Attorney for applicant
Reg. No. 52,264
Phone: 206-265-7817

Amgen Inc.
Law Department
1201 Amgen Court West
Seattle, WA 98119-3105
(206) 265-7000

Attachments

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date indicated below.

Date: Oct. 11, 2005 Signed: Janice M. Kerlson

Annotated Sheet Showing Changes

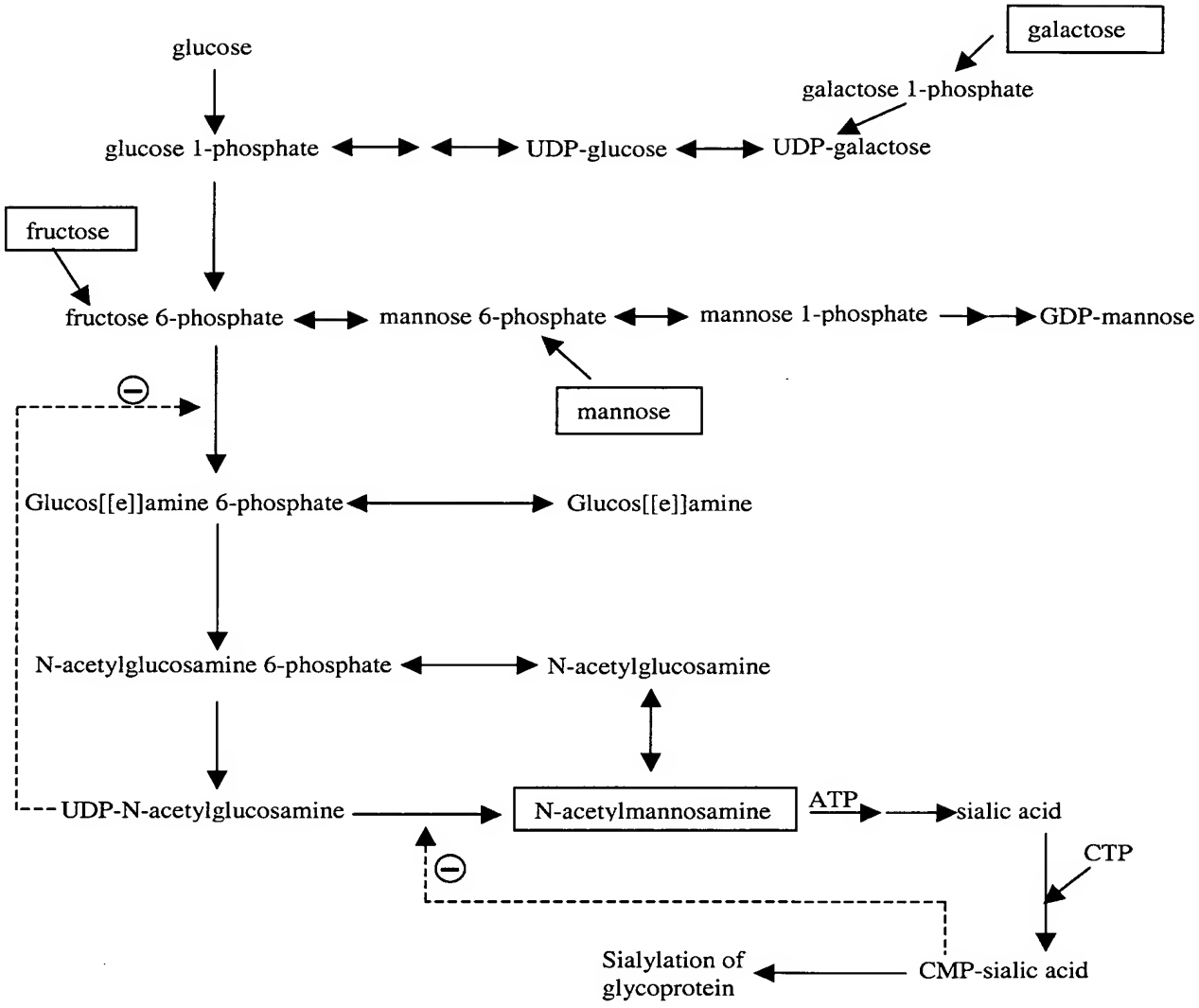

Figure 1